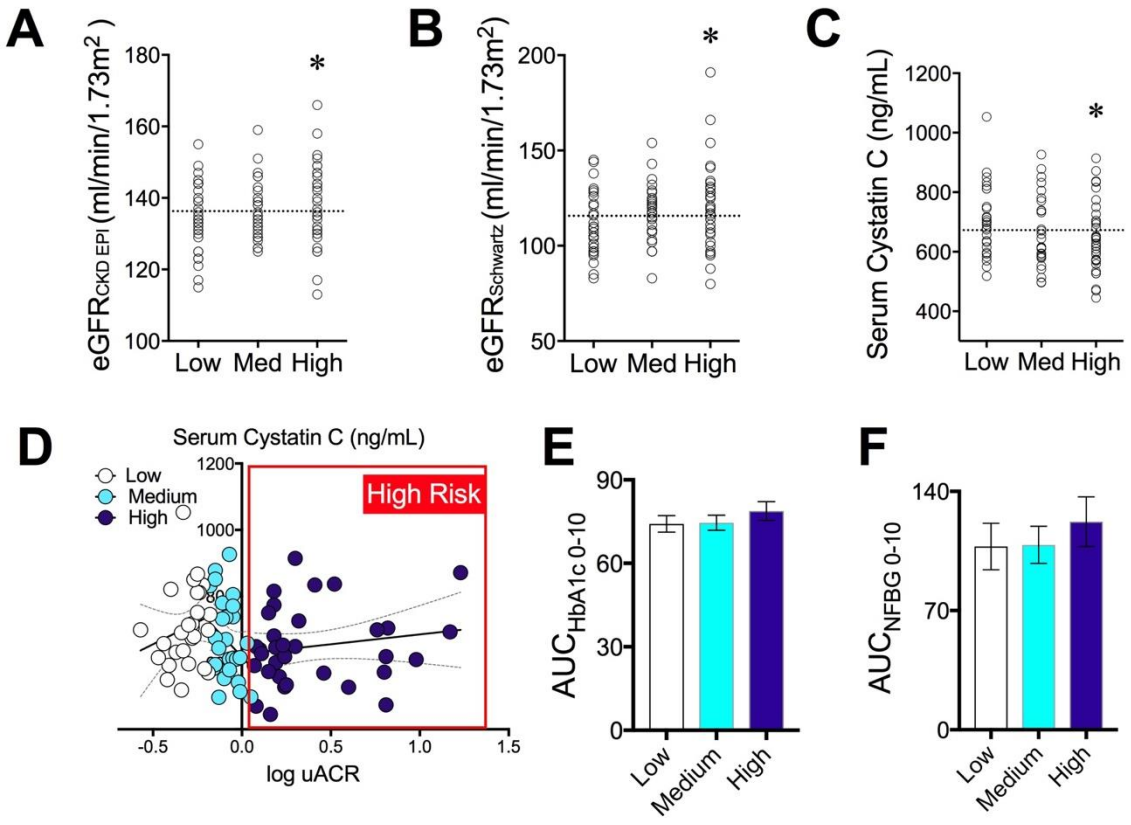
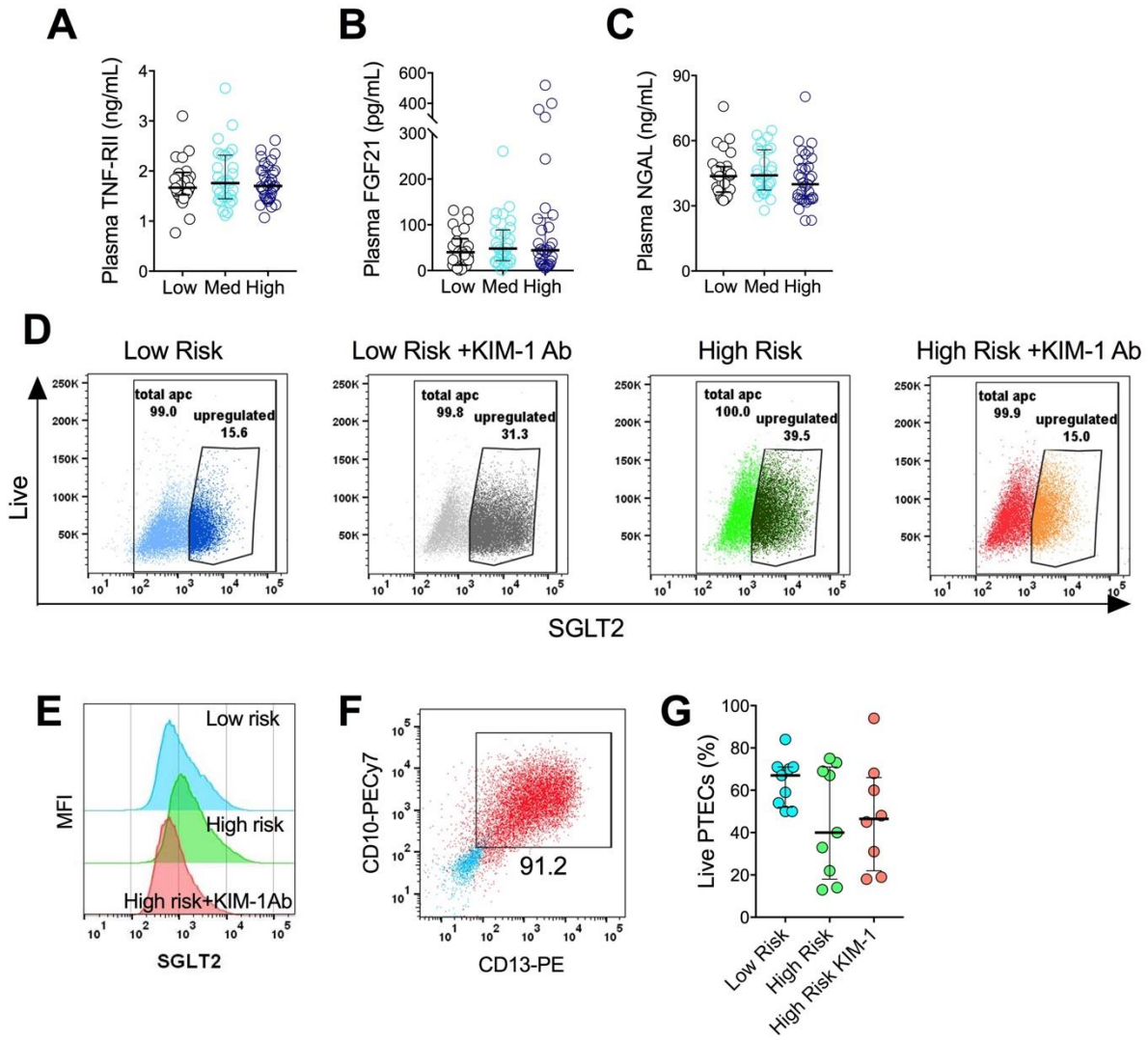
Forbes et al Supplementary Appendix; "T cell expression and release of kidney injury molecule-1 in response to glucose variations initiates kidney injury in early diabetes":

Parameters	**Control Healthy Tissue**	**Minimal Change Disease**	**1º FSGS**	**Diabetic Kidney Disease**
Age (years)	50.3±22.4	23.3±5.9	54.5±20.3	51.5±17.9
Sex *N*, (% female)	4 (0)	4 (25)	4 (50)	4 (25)
BMI (kg/m^2)	N/A	N/A	N/A	N/A
Random BG (mmol/L)	6.4±1.2	5.1±1.0	6.4±2.2	9.6±0.4
Diabetes Duration (years)	0	No Family History	No Family History	11.8±6.7
HbA_{1C} (%;mmol/mol)	No record (available for one of four patients)	No record	No record (available for one of four patients)	8.7±4.0
SBP (mmHg)	No record	132.5±12.6	138.8±13.1	142.5±8.7
Total Chol (mmol/L)	No record (available for one of four patients)	No record (available for two of four patients)	5.1±0.4	5.4±1.1
$eGFR_{CKD\ EPI}$ ($ml/min/1.73m^2$)	76.5±10.7	85.8±8.5	36.8±19.8	27.5±16.7
uPCR (g/mol)	No record (available for one of four patients)	686.3±640.3	244.3±171.7	684.8±491.5

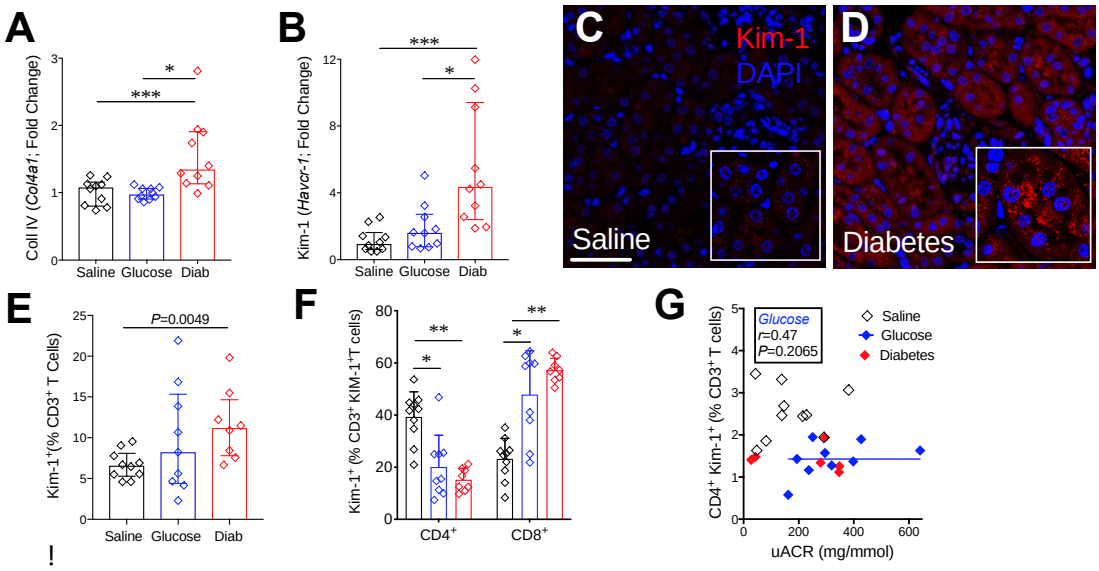
Suppl. Table S1. – Demographic and clinical characteristics of human kidney tissue donors. Control kidney tissue was obtained following Nephrectomy. Minimal Change Disease – immunofluorescence negative; IF/TA negative. Diabetic kidney disease – confirmed by the presence of Kimmelstiel-Wilson nodules, mesangial matrix expansion, immunofluorescence positive, IF/TA positivity. Focal Sclerosing Glomerulosclerosis (FSGS) – phenotypically not secondary FSGS; immunofluorescence negative; IF/TA positive. Minimal Change Disease – immunofluorescence negative; IF/TA negative. uPCR - urinary protein excretion/mol of creatinine. Data presented are Mean±SD.



Suppl. Fig. S1. Kidney function data in youth with varied risk for DKD. Youth (20.0±2.8 yrs old) with type 1 diabetes were stratified by risk for DKD using tertiles of urinary albumin:creatinine ratio (uACR; Low Risk , *N*=33; Medium Risk, *N*=33; High Risk, *N*=34). **A-C** Renal functional data plots of estimated glomerular filtration rate (eGFR) with the population mean shown as a dotted line using **(A)** the adult CKD-EPI equation; **(B)** the pediatric modified Schwartz equation; **(C)** the surrogate inverse marker serum cystatin C. **(D)** General linear regression plot of log uACR vs serum cystatin C corrected for age, sex, diabetes duration and HbA_{1C}. **E-F** Area under the curve (0-10 years) for historical **(E)** long term glucose control by HbA_{1C}; and **(F)** Random blood glucose concentrations (NFBG). **P*<0.05; ***P*<0.01 vs low risk group.



Suppl. Fig. S2. Biomarkers of kidney injury and proximal tubule cells exposed to plasma from youth at risk for DKD. Youth (20.0±2.8 yrs old) with type 1 diabetes were stratified by risk for DKD using tertiles of urinary albumin:creatinine ratio (uACR; Low Risk , *N*=33; Medium Risk, *N*=33; High Risk, *N*=34). **A-C** Plasma biomarkers were measured by ELISA **(A)** tumor necrosis factor receptor -2 (TNF-RII); **(B)** fibroblast growth factor -21 (FGF-21) and **(C)** Neutrophil gelatinase-associated lipocalin (NGAL) in all youth within the cohort. **D-G** Human healthy primary proximal tubule epithelial cells (PTEC) were exposed to plasma (4%) from youth with varied risk for DKD for 24 hours in the presence and absence of KIM-1 blockade (Ab). **(D)** Representative Flow cytometry dot plots of shift in SGLT2-APC expression in PTEC following exposure to 4% patient plasma. **(E)** Representative flow cytometry dot plot characterizing live kidney cells as human PTEC by dual expression of CD10 and CD13 after exposure to patient plasma; **(F)** Proportion of live PTEC after 24 hours exposure to plasma; **(G)** Flow cytometry histograms of SGLT2 expression in live PTECs at 24 hours (blue-Low Risk; green-High Risk+Control Ab; red -High Risk+KIM-1 blocking Ab.



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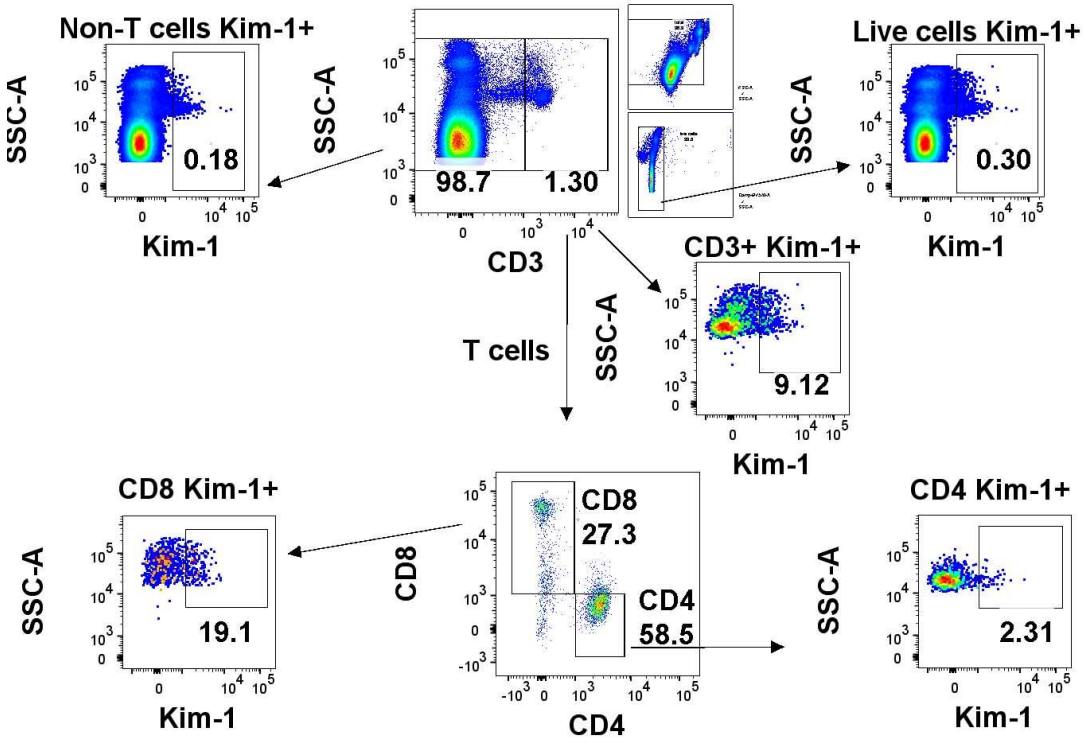
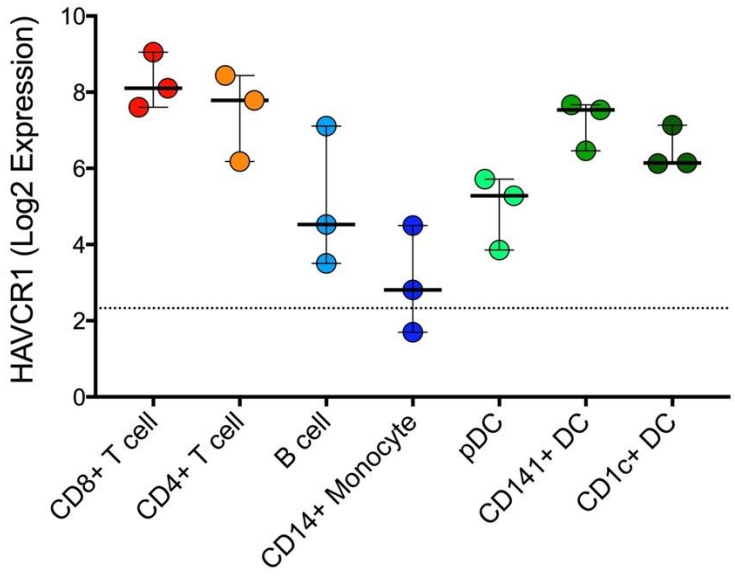
!	**Saline'** (*N*=10)!	**Glucose'** (*N*=10)!	**Diabetes'** (*N*=10)!
KW:BW!(mg/g)!	!!6.36±0.59!	!!6.41±0.66!	!!8.39±0.59***$^{\#\#\#}$!
Random!BG!(mmol/L)!	10.0±1.4!	9.8±1.4!	!!27.5±6.4***$^{\#\#\#}$!
AUC!Blood!Glucose!	60.5±5.3!	110.0±7.0***!	nd!
Plasma!Cystatin!C!(ng/ml)!	644.5±12.9!	595.0±94.8!	487.9±116.8**!

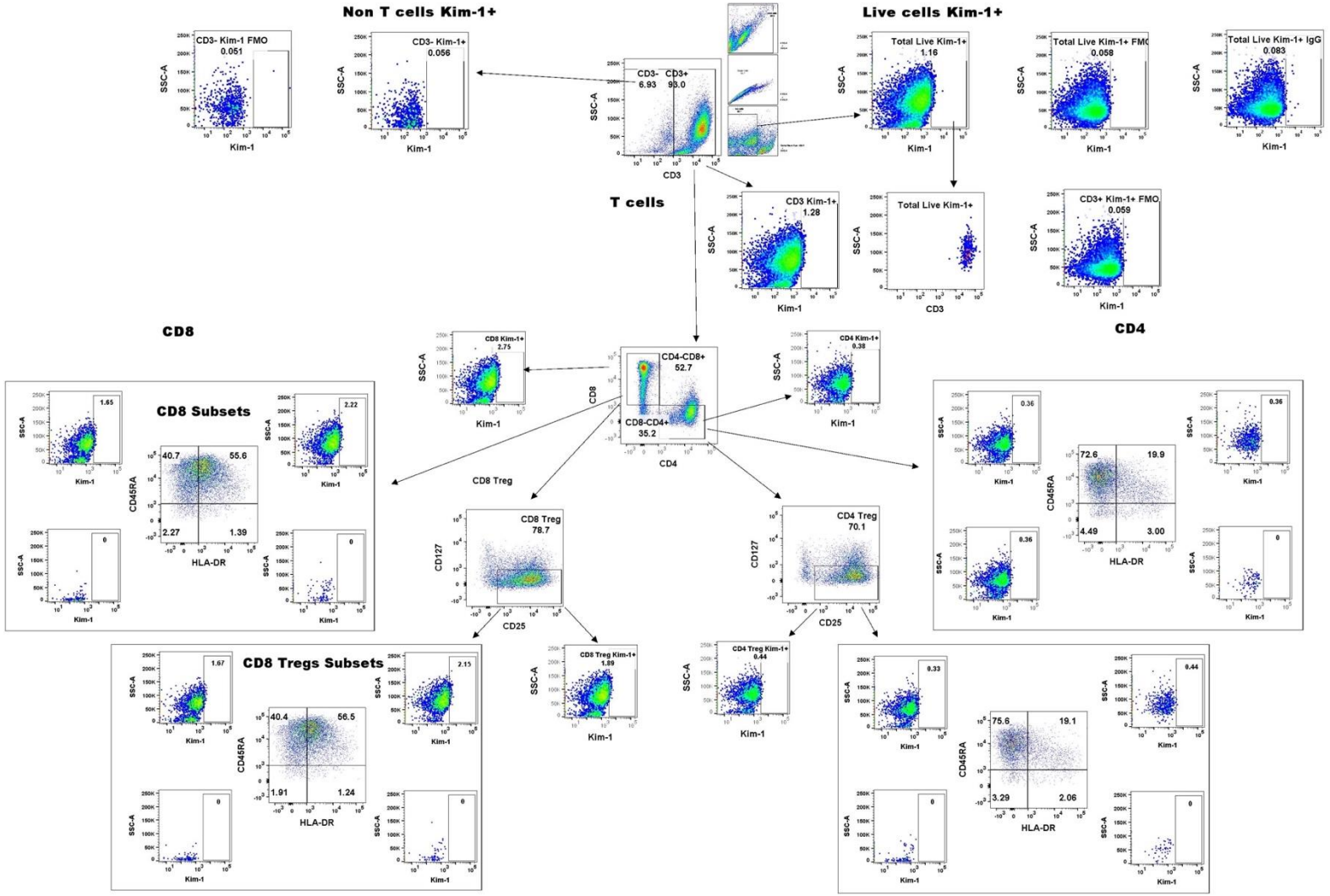
Suppl. Fig. 3. Glucose variability even in the absence of diabetes increases T cell Kim-1 expression in preclinical models. Male adolescent apolipoprotein E deficient mice **(***ApoE-/-;* 6 weeks of age) received four intraperitoneal (i.p.) injections of glucose (2g/kg; blue diamond symbols; *N*=10) or isovolumetric saline injections (saline; white diamonds; *N*=10), delivered two hours apart, to achieve plasma glucose variations which peaked between ~15-20mmol/L. **A-B** Kidney cortical gene expression by qPCR of **(A)** Collagen IV (*Col4a1*) or **(B)** Kim-1 (*Havcr1*). **C-D** Representative photomicrographs of kidney cortical Kim-1 (red) and nuclear (DAPI, blue) immunofluorescence in **(C)** saline injected or **(D)** diabetic mice. Scale Bar = 50 μm; x200 Magnification. **E-G** Flow cytometry analysis of live peripheral blood Kim-1 positive (Kim-1+) **(E)** T cells (Kim-1+CD3+); **(F)** CD4+ (Kim-1+CD3+CD4+CD8$^-$) and CD8+ (Kim-1+CD3+CD8+CD4$^-$) T cells as proportion of CD3+KIM-1+ T cells. Linear regression of **(G)** CD4+CD8$^-$Kim-1+; **(H)** Characteristics of *ApoE-/-* murine groups at the study end. Data are shown as mean SD or median (IQR) and tested using 1 way ANOVA/Tukey's post-hoc or Kruskal Wallis/Dunn's post-hoc testing. **P*<0.05; ***P*<0.01, ****P*<0.001 vs saline group, ###*P*<0.001 vs glucose group.



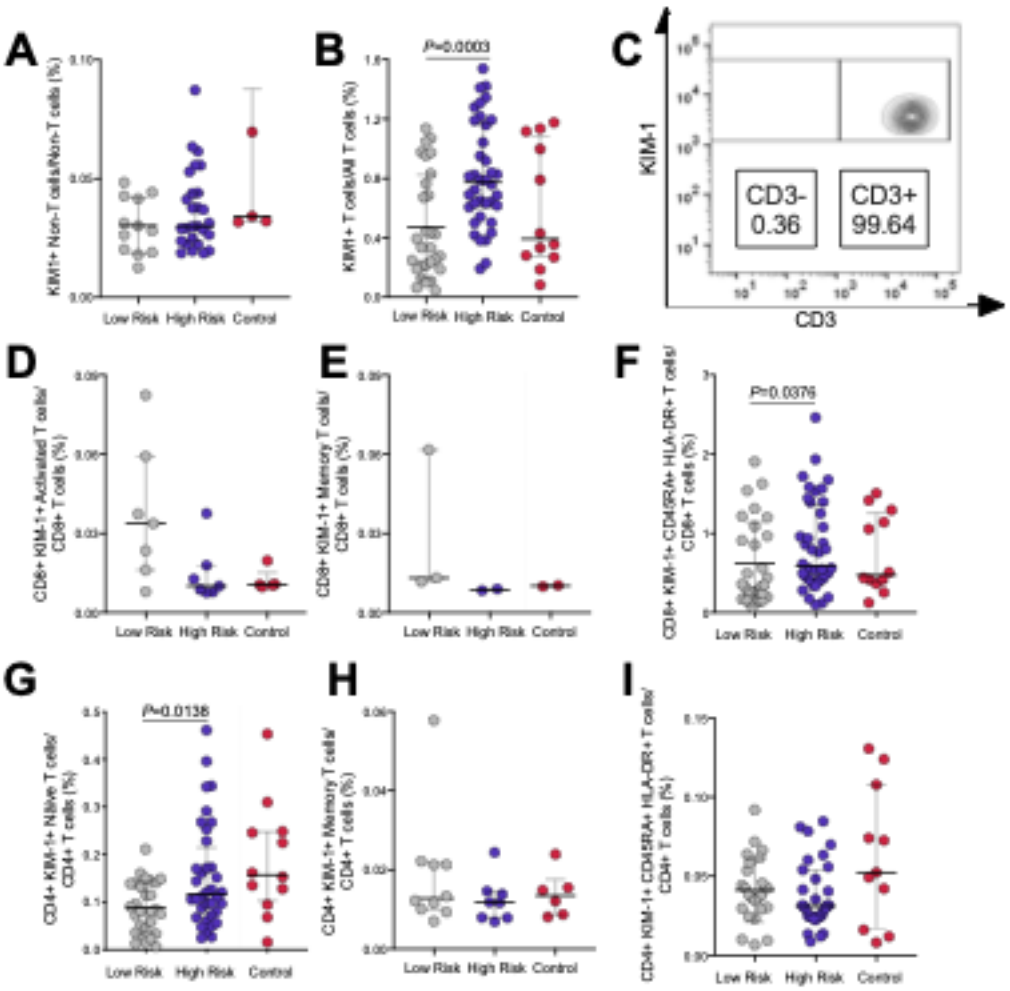
Suppl. Fig. S4. Gating strategy for KIM-1+ Peripheral Blood Mononuclear Cells in *ApoE*-/- mice at risk for DKD.



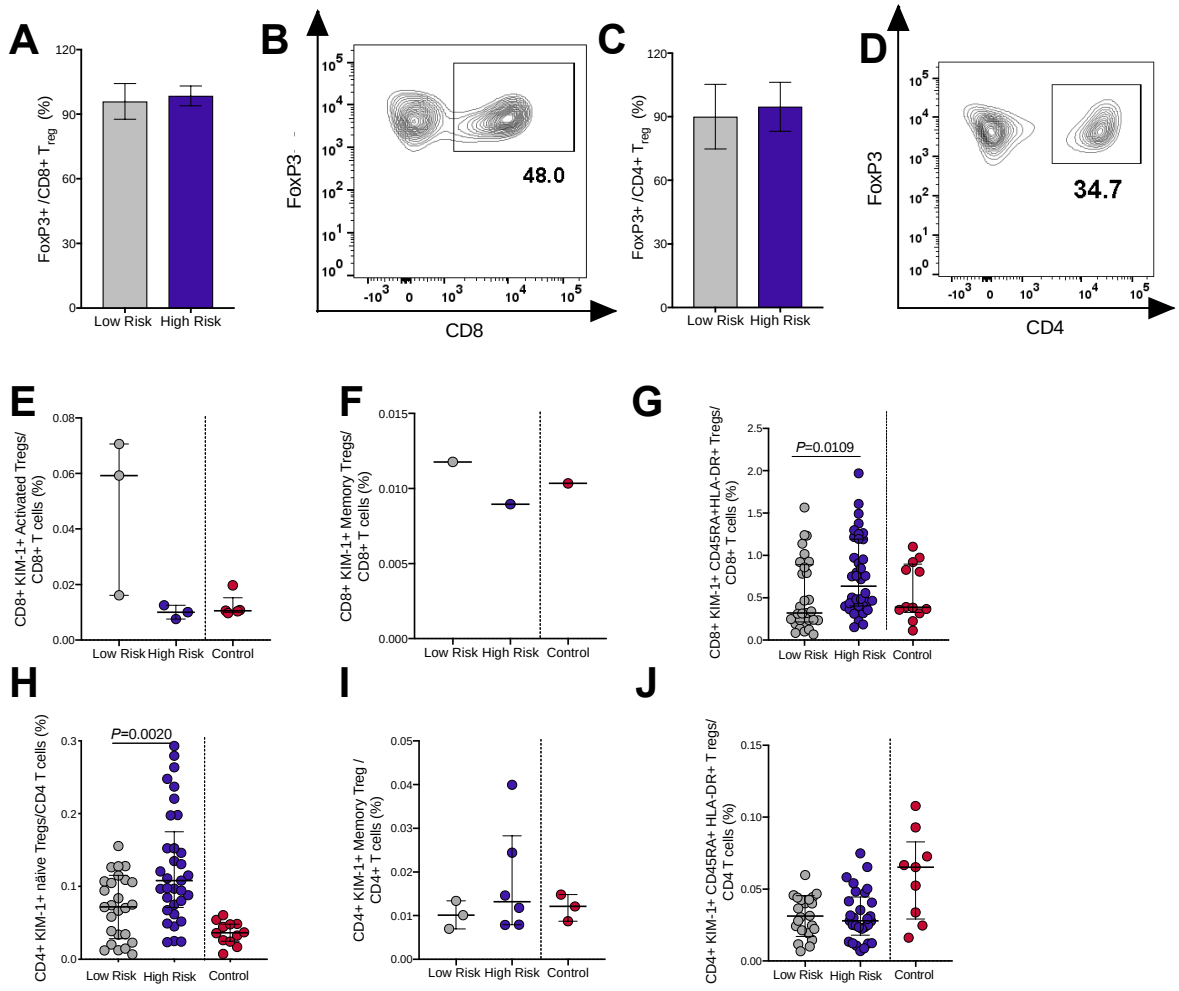
Suppl. Fig. S5. Peripheral blood mononuclear cell expression of *HAVCR1* (KIM-1 gene) in healthy human donors. Expression data was obtained from the gene expression omnibus (GEO) dataset GSE77671. Data were processed for background correction, normalization and log2-transformation within R and integrated into the Stemformatics platform [www.stemformatics.org] for visualization. *N*=30 donations from peripheral blood. The probe shown for HAVCR1 is A_23_P347610.



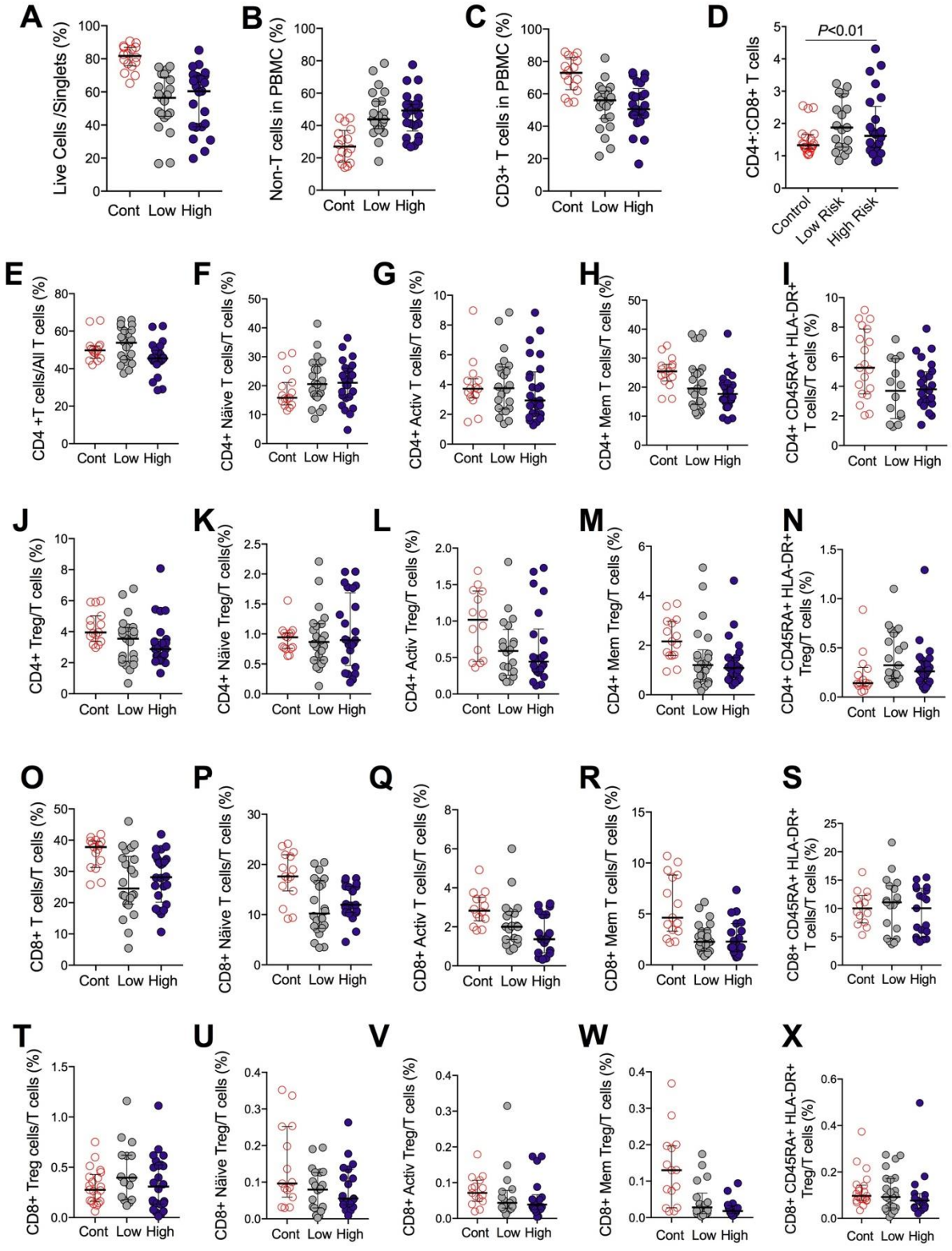
Suppl. Fig. S6. Gating strategy for Peripheral Blood Mononuclear Cells in youth at risk for DKD.



Suppl. Fig. S7. T conventional cell subset KIM-1 expression in youth at high risk for DKD. Youth (20.0±2.8 yrs old) with type 1 diabetes were stratified by risk for DKD using tertiles of urinary albumin:creatinine ratio (uACR; Low Risk , *N*=33; Medium Risk, *N*=33; High Risk, *N*=34). KIM-1 expression on live peripheral blood mononuclear cells (PBMCs) is shown for low and high risk groups. Circulating PBMC proportions of live KIM-1+ **(A)** $CD3^-$ KIM-1+ non-T cells and **(B)** CD3+KIM-1+ T cells. **(C)** Live KIM-1+ PBMC shown as a contour diagram. Live CD3+ T cells expressing CD4+ and CD8+ were sorted into specific subsets of **(D)** CD8+ activated T_{conv} ($CD3+CD8+CD4^-CD45RA^-HLA\text{-}DR+$); **(E)** CD8+ Memory T_{conv} ($CD3+CD8+CD4^-CD45RA^-HLA\text{-}DR^-$); **(F)** $CD3+CD8+CD4^-CD45RA+HLA\text{-}DR+$ T_{conv} double positive cells; **(G)** CD4+ näive T_{conv} ($KIM\text{-}1+CD3+CD4+CD8^-CD45RA^-HLA\text{-}DR^-$) T cells; **(H)** CD4+ Memory T_{conv} ($CD3+CD4+CD8^-CD45RA^-HLA\text{-}DR^-$); or **(I)** $CD3+CD4+CD8^-CD45RA+HLA\text{-}DR+$ T_{conv} double positive cells. $KIM\text{-}1+CD4+$ activated T_{conv} ($KIM\text{-}1+CD3+CD4+CD8^-CD45RA^-HLA\text{-}DR+$) T cells are not shown as they were present in negligible quantities; Data are shown as mean±SD or median (IQR) and tested using Student's T test or Mann Whitney U testing.



Suppl. Fig. S8. T regulatory cell subset KIM-1 expression in youth at high risk for DKD. Youth (20.0±2.8 yrs old) with type 1 diabetes were stratified by risk for DKD using tertiles of urinary albumin:creatinine ratio (uACR; Low Risk , *N*=33; Medium Risk, *N*=33; High Risk, *N*=34). KIM-1 expression on live peripheral blood mononuclear cells (PBMCs) is shown for low and high risk groups. **A-D** FOXP3 expression in **(A)** CD8+ T regulatory cells (T_{reg}); **(B)** CD8+ T_{reg} as a Contour plot; **(C)** CD4+ T_{reg} and **(D)** as a Contour plot in CD4+ T_{reg} cells. Circulating live CD3+KIM-1+ T_{reg} expressing CD4+ and CD8+ were sorted into specific subsets of **(E)** CD8+ activated T_{reg} (CD3+CD8+CD4$^-$CD-25+CD127$^{lo/-}$CD45RA$^-$HLA-DR+); **(F)** CD8+ Memory T_{reg} (CD3+CD8+CD4$^-$CD-25+CD127$^{lo/-}$CD45RA$^-$HLA-DR$^-$); **(G)** CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR+ double positive T_{reg} cells; **(H)** CD4+ näive T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA$^-$HLA-DR$^-$); **(I)** CD4+ Memory T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA$^-$HLA-DR$^-$); **(J)** CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR+ double positive T_{reg} cells. KIM-1+CD4+ activated T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA$^-$HLA-DR+) are not shown as they were present in negligible numbers. Data are shown as mean±SD or median (IQR) and tested using Student's T test or Mann Whitney U testing.



Suppl. Fig. S9. White blood cell subsets in peripheral blood from youth with type 1 diabetes and varied risk for DKD. Peripheral leukocytes were obtained from youth (20.0±2.8 yrs old) with type 1 diabetes with varying risk for DKD (uACR; Low Risk , *N*=33; Medium Risk, *N*=33; High Risk, *N*=34). **(A)** Live cells were quantified using flow cytometry into **(B)** Non T cell subsets ($CD3^-$); **(C)** T cells (CD3+); **(D)** CD4+:CD8+ T cell ratio; **(E)** CD4+ conventional T cells ($CD3+CD4+CD8^-$) and **F-I** the CD4+ conventional (T_{conv}) T cell subsets of **(F)** naïve T_{conv} ($CD3+CD4+CD8^-CD45RA+HLA\text{-}DR^-$); **(G)** activated T_{conv}

(CD3+CD4+CD8$^-$CD45RA$^-$HLA-DR+); **(H)** memory T_{conv} (CD3+CD4+CD8$^-$CD45RA$^-$HLA-DR$^-$) and **(I)** CD3+CD4+CD8$^-$CD45RA+HLA-DR+ T_{conv}. **(J)** CD4+ T regulatory (T_{reg}) cells (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$) sorted into **K-N** the CD4+ T_{reg} subsets of **(K)** naïve T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR$^-$); **(L)** CD4+ activated T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA-HLA-DR+); **(M)** CD4+ memory T_{reg} (CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA$^-$HLA-DR$^-$) and **(N)** CD3+CD4+CD8$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR+ T_{reg}. **(O)** CD8+ T_{conv} cells (CD3+CD8+CD4$^-$) sorted into **P-S** the CD8+ T_{conv} subsets of **(P)** naïve T_{conv} (CD3+CD8+CD4$^-$CD45RA+HLA-DR$^-$); (**Q)** activated T_{conv} (CD3+CD8+CD4$^-$CD45RA$^-$HLA-DR+); **(R)** memory T_{conv} (CD3+CD8+CD4$^-$CD45RA$^-$HLA-DR$^-$) and **(S)** CD3+CD8+CD4$^-$CD45RA+HLA-DR+ T_{conv}. **(T)** CD8+ T regulatory (T_{reg}) cells (CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$) sorted into **U-X** the CD8+ T_{reg} subsets of **(U)** naïve T_{reg} (CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR$^-$); **(V)** CD8+ activated T_{reg} (CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$CD45RA-HLA-DR+); **(W)** CD8+ memory T_{reg} (CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$CD45RA$^-$HLA-DR$^-$) and **(X)** CD3+CD8+CD4$^-$CD25+CD127$^{lo/-}$CD45RA+HLA-DR+ T_{reg}. Data are shown as mean SD or median (IQR) and tested using 1 way ANOVA/Tukey's post-hoc or Kruskal Wallis/Dunn's post-hoc testing.